STUDY ON SERUM ZINC LEVEL IN ACUTE DIARRHOEAL DISEASES AMONG

100 CHILDREN ADMITTED AT RMH, THANJAVUR.

DISSERTATION SUBMITTED FOR

M.D.DEGREE EXAMINATION

BRANCH – VII PAEDIATRIC MEDICINE

THANJAVUR MEDICAL COLLEGE, THANJAVUR

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

MARCH - 2009

DECLARATION

I declare that this dissertation Entitled "**STUDY ON SERUM ZINC LEVEL IN ACUTE DIARRHOEAL DISEASES AMONG 100 CHILDREN ADMITTED AT RMH"** has been conducted by me at the Department of Paediatrics in the Diarrhoea training and treatment unit, Government Raja Mirasudhar Hospital, Thanjavur attached to Thanjavur Medical College, under the guidance and supervision of PROF.DR.V.ILAKKUMI, M.D.,DCH. & PROF.DR.SELLARAMAN, M.D., DCH. It is submitted in partial fulfillment of the award of the degree of M.D. (Paediatrics) for the March 2009 examination to be held under the Tamil Nadu DR.M.G.R. Medical University, Chennai. I have not submitted this previously for the award of any degree or diploma from any other university.

CERTIFICATE

This is to certify that "**STUDY ON SERUM ZINC LEVEL IN ACUTE DIARRHOEAL DISEASES AMONG 100 CHILDREN ADMITTED AT RMH"** is a bonafide work done by **DR. S. RAMYA, M.B.B.S.,** under the guidance and supervision of **PROF. DR. V. ILAKKUMAI, M.D.D.C.H.** It is submitted in partial fulfillment of the degree of **M.D BRANCH VII PAEDIATRIC MEDICINE** for the **March 2009 examination to be held under the Tamilnadu Dr. M.G.R Medical university, Chennai.**

HEAD OF THE DEPARTMENT **DEAN**

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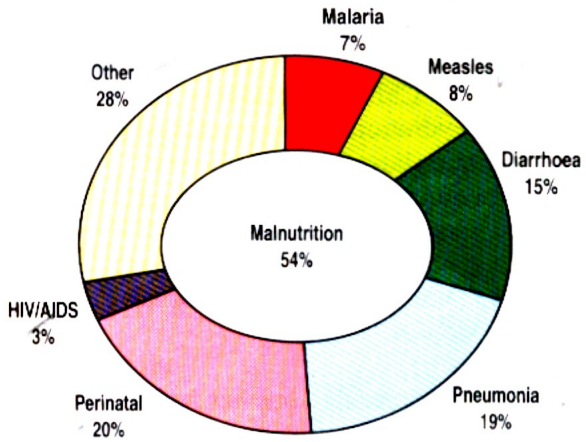
CONTENTS

Introduction

Over the last 3 decades the annual number of deaths among children less than 5 years of age has decreased by almost a third. However this reduction has not been the same throughout the globe. Every year more than 10 million children die in developing countries before they reach their 5th birthday.

Projections based on the 1996 analysis (1) indicate that common childhood illnesses will continue to be a major contributor to child death throughout the year 2020 unless greater efforts are made to control them.

Distribution of 10.5 million deaths among children less than 5 years old in all developing countries 1999.



Diarrhoea

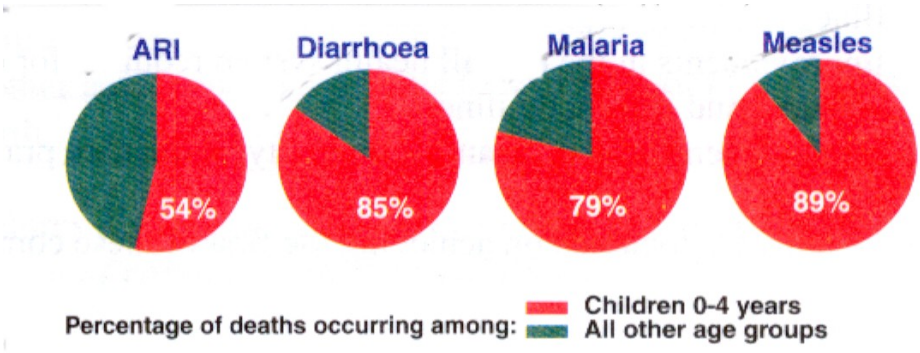
Defined as the abrupt onset of abnormally high fluid content in the stool (more than the normal value of approximately l0ml/ kg/ day) characterized by increased frequency of bowel movements.

WHO/UNICEF defined acute diarrhoea as an abrupt attack of sudden onset which usually last 3 to 7 days but may last upto 10 – 14days.

Acute diarrhoea is a major cause of morbidity and mortality among infants and preschool children. Though the mortality rate for children under five suffering from diarrhoea has fallen drastically from 4.5 millions deaths / year in 1979 to 1.6 million deaths in 2002. Acute diarrhoea continues to exert a high toll on children in developing countries.

For children aged under 5years in developing countries a median of 3.2 episodes of diarrhoea occurred per child year and estimates of mortality indicate that 4.9 children per 1000 per year died in the developing regions as a result of diarrhoeal illness in the first five year of life (2).

Proportion of global burdern of selected diseases borne by children under 5 year (Estimates, year 2000).



According to WHO estimates for the year 2002 there were about 1.798 million deaths and 61.96 million DALY lost due to diarrhoea (3). This makes a strong case for introducing new strategies to significantly reduce child mortality and morbidity due to diarrhoeal illnesses.

Two recent advances in managing diarrhoeal diseases – newly formulated oral rehydration salts (ORS) containing lower concentration of glucose and salt and success in using zinc supplementation can drastically reduce the number of child deaths.

The prevalence of zinc deficiency has been estimated at approximately 20% world wide (4) but might be higher in certain population.

Zinc deficiency causes reduced growth and stunting (5) reduced immuno competence (6) and improved psychomotor development.

Zinc status is defined by serum zinc levels in the international reference values between 11.6μ mol/L and 23.0 μ mol/L (7) zinc level below 10.71 μ mol/L in samples of blood serum are defined by WHO as zinc deficiency (8).

Review of Literature

Zinc is present in all organs, tissues, fluids and secretions of the body. Lean body mass zinc concentration is 30μg/gram, chiefly distributed in skeletal muscles (60%) and bones (30%).

Plasma zinc accounts for only 0.1% of total body zinc. The element is necessary for ribosome stabilization.

Zinc is critical for functioning of biomembranes. Zinc participates in the synthesis and degradation of carbohydrates, lipids, protein and nucleic acid.

Zinc is part of several enzyme systems of the body e.g. carbonic anhydrase. Recently zinc has been shown to play a role in the process of genetic expression**.**

Zinc has been recognized as an essential element, which takes part in metabolism. Zinc influences cell division, growth, DNA and RNA metabolism (9), growth, development sexual maturation and the immune system.

Zinc concentrations of plasma, blood cells, hair and urine decrease in severe deficiency states. Infection, fever, other stresses and pregnancy also tend to reduce zinc levels. Zinc fortification of diets has been found to be associated with improvements in dark adaptation, conjunctival integrity, immunno competence, growth rate and reduced perinatal mortality .

Zinc is considered to have an important immuno-regulatory effect (10) on lymphocytes, lymph tissue, neutrophils, macrophages, mastocytes and platelets. Zinc deficiency is among the 10 most important factors that lead to increased mortality and morbidity in developing countries. (11,12).

Insufficient intake of zinc is a leading cause for zinc deficiency. This was underlined in the 2002 annual health report of WHO, which gave directions for introducing zinc additions into daily nutrition. Clinical manifestations of zinc deficiency in early childhood can lead to acute or chronic diarrhoea with malnutrition, mental disorders and behavioral problems. With age, one can observe alopecia, growth retardation, skin lesions and common infections in children with zinc deficiency, (12).

Zn-combating diarrhoea

It is estimated that 3,000 of the hundreds of thousands of proteins in the human body contain zinc prosthetic groups, one type of which is so-called zinc finger. In addition, there are over a dozen types of cells in the human body that secrete zinc ions, which are also new entering the domain of neurotransmitter cells in the salivary gland, prostate, immune system and intestine are other types that secrete zinc.

The benefit of zinc in diarrhoea is mediated via a variety of pathways including stabilization of the epithelial barrier and regeneration of the intestinal mucosa (13 – 21)

Restoration of brush border enzymatic (22 – 28) function, inhibition of CAMP induced chloride secretion blocking baso – lateral potassium channels.

Sources :

Animal foods such as lean red meat and pork are rich source of zinc. Refined diet low in cereal fiber and having a phytate zinc molar ratio < 5 have a high bioavailability of zinc. Cheese, whole wheat, nuts and legumes also provide zinc.

Bioavailability :

Low calcium and high protein intake promote absorption and retention of zinc. Animal protein including milk appear to promote zinc release and bioavailability from its phytate complex. Phytate (present in bran, whole grain cereals , and legumes) and high iron intake inhibit zinc absorption. Iron has little effect on zinc absorption from a complex meal. Fats tend to dilute zinc from the total diet.

Average adult Indian vegetarian diet usually contain 16mg of zinc so that 10% of this (1.6 mg) is available.

Mechanisms of action of zinc

Zinc is a micronutrient widely present in the human body, and it is involved in several metabolic processes, immune functions, and mucosal trophism. Zinc reduces ion secretion and nitric oxide synthesis and improves appetite, absorption, regeneration of enterocytes, restoration of enteric enzymes and intestinal permeability and humoral and cellular immunities (29,30,31- 35).

In vitro, zinc promotes ion absorption and prevents the secretary effect induced by heat – labile vibrio – cholera enterotoxin by directly interacting with intracellular cyclic adenosine monophosphate (36).

However, zinc can not counter the secretion induced by heat stable E coli enterotoxin.

Zinc protects the enterocytes from damage induced by E. coli (Entero Toxigenic E. coli) by providing the disruption of membrane integrity and altered permeability, reducing bacterial adhesion, blocking the invasiveness, and counteracting cytokines alteration (increase of IL – 8 and TNF – α ,decrease of transforming growth factor β (37).

In children with shigellosis administration of elemental zinc (20mg for 2 wk) significantly increase the proliferation response of lymphocytes and the production of specific immunoglobulins (invasion plasmid - encoded antigen) (38). zinc also may improve weight gain because of its effects on growth through growth hormone and insulin like factor – 1 (39,40).

Studies of immune functions in experimental human models.

Zinc deficiency in humans was described only in early 1960s. (41,42).

Thymulin is a thymus specific hormone which require the presence of zinc for its biological activity to be expressed (43,44).

Thymulin binds to high affinity receptors on T cells, induces several T – cell markers, and promotes T – cell function including allogenic cytotoxicity, suppressor function and IL – 2 production (43, 44).

Mild deficiency of zinc leads to an imbalance of T – helper (Th_1) and T – helper 2 (Th2) functions, decrease the recruitment of T naive cells (CD4 + CD4 5RA+), and decreases the percentage of CD73 + cells in the CD8 + subset that are precursors to cytotoxic T lymphocytes (45,46).

Zinc is essential for the activity of thymulin, zinc may possible be intrinsically involved in the development of haematopoietic stem cells in the thymic micro environment (43,44)

Effect of zinc deficiency on immune function in experimental human models.

1. Thymulin activity decreased corrected by both in vivo and in - vitro zinc supplementation
2. T – cell subpopulation studies.

   CD_4 + to CD8 + Ratio decreased.

   CD_4 + $CD_4$5RA+ to CD + $CD_4$5RO + Borderline
3. Th_1 cytokines both cytokines decreased.

   IL – 1

   IFN - γ
4. Th_2 cytokines No change

   IL – 4, IL – 6, IL – 10.
5. NK cell lytic activity Decreased

Precursors of cytotoxic T lymphocytes

CD8 + CD73 + Decreased.

Zinc in acute diarrhea.

Zinc supplementation during diarrhoea is recommended in developing countries by the world health organization (WHO) and the united Nations childrens fund (UNICEF) based on several trials demonstrating (47) a significant effect of zinc (as zinc acetate, gluconate, or sulfate alone or added to other vitamins, micronutrients or ORS) in reducing the duration, severity, and recurrence of diarrhoea in immunocompetent and in severely malnourished or immune – deficient children (29, 30, 48, 49).

Insufficient intake of zinc has been reported in patients with enteropathy and malnutrition but also in healthy children in Europe and United states (50,51).

During diarrhoea, zinc deficiency may also manifest to an increased requirement related to intestinal loss (52, 53). However, zinc supplementation is currently not considered in developed countries and is recommended in developing countries or in malnourished patients.

Zinc In the Treatment of Diarrhoea

In the trials subjected to pooled analysis (54) zinc supplemented children had 16% faster recovery and zinc treatment also resulted in a 20% reduction in the odds of acute episodes lasting > 7 days.

The study by Bhatnagar et al (55) is of interest as it was hospital based, acute diarrhoea with involved cases of acute diarrhoea with dehydration and measured impact on stool output was reduced by 31% than in placebo group.

Antibiotic use was less in areas where 20mg zinc was introduced with ORS and the ORS use rate increased by 50% in comparison with the group which didn't receive zinc (56).

Zinc and prevention of diarrhea

Randomized controlled trials from developing countries have shown the efficacy of zinc in preventing intestinal infections and decreasing the incidence, duration, recurrence of diarrhea, and the use of antibiotics. (57 – 61)

Zinc reduces the risk not only for diarrhoea but also for pneumonia, malaria, and overall mortality (62)

Evaluating child health strategy mainly focusing on reduced mortality, zinc fortification, and supplementation (in adjunct to ORS) has been recently considered by the WHO as an efficacious and cost - effective intervention (63)

Food sources of zinc for infants

The feasibility and potential of a local non fortified food – based approach for preventing onset of zn deficiency in midinfancy are challenging. An infant of 6-8 months is in the critical transition period of infant feeding. Breast milk, perhaps augmented by the release of modest neonatal stores in early infancy (64), Provides sufficient zn for the first few months of life. However, as lactation progresses, the physiologic decline in breast milk zn concentration is notable (65)

By 7 months –postpartum, the zn concentration in human milk is <1 mg/L (65) and the intake of Zn from breast milk by the exclusively breast – fed 7 – months infant is only 0.5 – 0.6 mg regardless of mother's zn status (65, 66).

Complementary foods in developing countries are typically limited almost entirely to plant foods, Zn concentrations in even the most favorable plant foods are inadequate to meet requirements(67)

This problem is compounded by unfavourable bio availability attributable to the inhibitory effect of phytate (68)

Plant foods with the most favourable Zn concentrations notably grains and legumes also have the highest phytate concentrations challenging research is now being directed to bio fortification of grains with Zn (69) and to lowering phytate. (70).

Micronutrient fortification of food staples provided a partial solution to achieving adequate Zn in plant foods. However, these fortified foods will not reach all older infant/ toddlers especially the millions of rural poor. (71) similar constraints are encountered with the availability of sprinkles yet to be shown efficacious in preventing or managing Zn absorption/ deficiency. There remains a compelling current and long term need for locally produced non fortified complementary foods providing adequate Zn. Animal source foods, especially meats including organ meats, not only contain the highest concentrations of Zn (72) but provide zinc in a bioavailable form.

Side Effects of Zinc

At the recommended doses of 10mg in the early months of life and 20 mg/d for children older than 6 months, No study has reported negative or Side effects of zinc administered alone (in sachet or capsule or added to vitamins or ORS in severely malnourished patients or those with the

human immunodeficiency virus (29,49); higher doses such as 15 mg/d in infant and 30 mg/d in children (three times the recommended dietary allowance (30).

Conversely, a zinc load may cause an active secretion of ions in the intestinal lumen(36)

A theoretical alteration of copper absorption, relevant in malnourished children, was not reported with zinc supplementation for 2 weeks (30).

Therapeutic compliance was considered as good in all studies with 84% adhesion to treatment in one study (47) but only about 50% in another trial (73)

Assessment of severity of Dehydration

Dehydration is the commonest and life threatening consequence of diarrhea. Young children are more susceptible to develop dehydration due to limited urinary concentration capacity of the kidneys, more insensible losses of water through skin and lungs owing to large surface area and rapid breathing, and their dependence on adults to replace their fluid losses

Loss of water and electrolytes in the diarrheal stool results in depletion of the ECF volume, electrolyte imbalance and clinical manifestation of dehydration. The first symptom of dehydration appears

after fluid loss of 5 percent of body weight. When fluid loss reaches 10 percent, shock often sets in, and the cascade of events that follows can culminate in death unless there is immediate intervention to rehydrate.

Assessment of hydration Status in a Patient with diarrhea

Clinicalsigns			
general condition	Well, alert	Restless, irritable	Lethorgic (or) unconscious
Eyes	Normal	Sunken	Sunken
Thirst	Drinks normally, Not thirsty	Drinks eagerly Thirsty	Drinks poorly Not able to Drink.
Skin Pinch	Goes back 'Quickly'	Goes back ' Slowly'	Goes back 'Very Slowly'
Decide Hydration status	The Patient has 'NO SIGNS OF DEHYDRATION'	If the patient has two (or) more signs, there is 'SOME DEHYDRATION'	If the patient has two (or) more signs, there is 'SEVERE DEHYDRATION'

Aim of the study

This present study was aimed,

To assess the serum zinc level in children with acute diarrhea and to correlate it with

i) Anthropometry

ii) Type of dehydration

iii) Recovery pattern of diarrhea.

Methodology

This hospital based purposive study conducted in diarrhoea training and treatment unit at Govt Raja Mirasudhar Hospital , attached to Thanjavur Medical College during the period between September 2007 to September 2008.

This hospital serves as a referral hospital for Thanjavur and adjacent districts mostly meeting out the health needs of rural agricultural population

The study population was choosen from the inpatients of the DTTU of this hospital

Inclusion criteria

Children between 6 months to 5 years of both sexes admitted with acute diarrhea.

Exclusion criteria

- Children with persistent diarrhea, recurrent diarrhea and chronic diarrhea
- Children with malnutrition due to other causes like cardiac, hepatic, metabolic, renal and degenerative diseases.
- Children who had micronutrient supplementation.

Eligible children were enrolled in the study after obtaining a informed consent form the parents/ Guardian, explaining the details of study procedure .

This study protocol was reviewed and approved by the ethical review committee.

Children were subjected to complete physical examination assessments of dehydration and anthropometry.

Anthropometry is a simple valuable tool and the gold standard for evaluating the nutritional status.

Weight

Weight was measured using a beam scale of salter type scale with pants in which the child was placed. The beam was properly balanced and moved freely when at rest and the pointer was on zero. The scale was set on a flat horizontal surface.

The shoes were removed and children were weighed with as little clothing as customs permitted. The child was not in contact with any other object.

Weight was either read directly or by balancing the beam depending on the type of scale. The result was read only after the beam reached the balance point or the pointer became motionless.

If children were restless double weighing was done. As accuracy was less satisfactory this was used as a last resort only.

The scale was checked with standard weight and zero error was corrected to the nearest value of ± 20 gms.

Height

Below the age of 2 years a horizontal measuring rod (or) infantometer was used. Height measured in lying down posture was called length. Length measurement needed two people. Shoes were removed & child was placed on a flat surface. One person preferably the mother maintained the top of child's head against the fixed vertical head board with the child's eyes directed upwards. The other persons firmly pressed the knees together and down so that they touched the horizontal surface and then moved the mobile foot board so that if touched the heels when the feet were at right angle. Accuracy was adjusted to the nearest 0.5cm. Beyond the age of two years, a vertical measuring rod or stadiometer was used. The child was made to stand bare foot and the heels, buttocks, shoulders & occiput touching the wall and looking straight ahead. The chin was made to be straight (in Frankfurt planes). The observer read the measurement directly after lowering the cursor or placing a horizontally held book or wooden board in order to touch the

top of head. The hair flattened and the accuracy measured to the nearest 0.5cm.

Both height and weight were recorded by a single observer.

Head circumference

While measuring head circumference the maximum occipito frontal circumference was measured by placing the flexible non stretchable tape firmly over the most prominent region of the occiput and frontal crests. The measurement was made accurate to the nearest of 0.1cm.

A base line assessment including detailed physical examination was performed at the time of enrollment.

For dehydrated children Anthropometry was repeated after hydration children were treated with standard diarrhoea treatment protocol. Within 24 hour of admission 3 ml of venous blood was collected via venipuncture using plastic tubes carefully washed to make them zinc free. The blood samples were transported to lab where serum zinc concentration were measured using calorimetric Method. A Certified trace element control serum was used daily to ensure accuracy and precision. All lab analyses were blinded to diarrhoea and intervention status of the children sampled.

COLORIMETRIC METHOD

ZINC METHODOLOGY

Principle

Zinc in an alkaline medium reacts with Nitro – PAPS to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of zinc present in the sample.

$$\text{Zinc} + \text{Nitro – PAPS} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{Purple Coloured complex}$$

Normal reference values

Serum	60 – 120 µg/dl
Urine	100 – 1000 µg/24 hrs.

It is recommended that each laboratory establish its own normal range representing its patient population.

CONTENTS	25 ml	75 ml
L1 : Buffer Reagent	20 ml	60 ml
L2 : Colour Reagent	5 ml	15 ml
S : Zinc Standard (200 µg/dl)	2 ml	2 ml

STORAGE / STABILITY

Contents are stable at 2 – 8^0 C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent :

Pour the content of 1 bottle of L2 (Enzyme Reagent 2) into 1 bottle of L1 (Enzyme Reagent 1). This working reagent is stable for atleast weeks when stored at 2 – 8^0 C.

Alternatively for flexibility as much of working reagent may be made as and when desired mixing together 4 parts of L1 (Enzyme Reagent 1) and 1 part of L2 (Enzyme Reagent 2) Alternative 0.8 ml of L1 and 0.2 ml 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample Material

Serum (Free from Hemolysis).

Zinc is Reported to be stable in serum for 7 days at 2-8^0 C.

Procedure

Wavelength/ Filter	:	570 nm (Hg578 nm) / Yellow
Temperature	:	R.T
Light Path	:	1 cm

Pipette into clean dry test tubes Labeled as Blank (B), Standard (S) and Test (T).

Addition Sequence	B (ml)	S (ml)	T (ml)
Working Reagent	1.0	1.0	1.0
Distilled water	0.05	-	-
Zinc Standard (s)	-	0.05	-
Sample	-	-	0.05

Mix well and incubate at R.T (25^{0}C) for 5 min. Measure the absorbance of the standard (Abs S), and Test Sample (Abs. T) against the Blank, within 20 Min.

Calculation

$$\text{Zinc in } \mu g/dl = \frac{\text{Abs. T}}{\text{Abs. S}} \times 200$$

Linearity

This Procedure is linear upto 700 µg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using an appropriate dilution factor.

System Parameters			
Reaction	: End Point	Interval	: …
Wavelength	: 578 nm	Sample Vol.	: 0.05 ml
Zero Setting	: Reagent Blank	Reagent Vol	: 1.00 ml
Incub . Temp	: R.T.	Standard	: 200 µg/ dl
Incub . Time	: 5 min.	Factor	: …
Delay Time	: …	React. Slope	: Increasing
Read Time	: …	Linearity	: 700 µg/ dl
No. of read	: …	Units	: µg/ dl

Follow up :

The children were followed up daily with a thorough history and detailed examination till the child recovered.

Recovery was defined as passage of one semisolid stool (or) no loose stool in the previous 18 hours.

The findings were recorded in Proforma.

STASTICAL ANALYSIS

Data has been entered in excel and analysed using SPSS PC + and EPI info 2000 statistical software and descriptive analysis was used.

The results were analysed by using ANOVA (comparison of means) & chi – square test.

DESCRIPTIVE STATISTICS

	N	Minimum	Maximum	Mean	Std. Deviation
AGE	100	6.00	60.00	16.8400	13.8014
WEIGHT	100	4.50	17.00	8.6730	2.5855
HEIGHT	100	59.00	105.00	75.1450	10.1440
ZINC	100	14.10	124.80	71.9610	25.9002
Valid N (listwise)	100				

Serum Zinc and Sex Distribution

SEX	TOTAL	MEAN AGE	95% CI	SD
MALE	68	16.4 (6 to 60)	13.3 – 19.5	12.7
FEMALE	32	17.7 (6 to 60)	11.9 – 23.5	16.06

P = 0.665 NS

68 were male children and 32 were female children

ZINC

SEX	Mean	N	Std Deviation
Male	71.1838	68	25.5391
Female	73.6125	32	26.9002
Total	71.9610	100	25.9002

P = 0.664 (NS)

Mean zinc level among male children was 71.1838µg/dl; Mean zinc level among female children was 73.6125 µg/dl. The difference observed was statistically not significant (P value 0.665).

SERUM ZINC LEVEL AMONG THE STUDY POPULATION

	Frequency	Percent
N	70	70.0
L	22	22.0
VL	8	8.0
Total	100	100.0

Zinc level

N – Normal 60 – 120µg/dl
L – Low 31 – 60µg/dl
VL – Very low <30µg/dl

ZINC LEVEL	Mean	N	Std Deviation
N	84.3200	70	19.3488
L	49.8955	22	6.2889
VL	24.5000	8	6.8076
Total	71.9610	100	25.9002

P< 0.0005 (Significant)Serum zinc level was low in 22% of the study subject, and very low in 8% of them, the difference observed was also statistically significant P< 0.0005.

SERUM ZINC LEVEL AND ANTHROPOMETRY

zinc level in relation to weight as per IAP classification

		Frequency	Percent
Valid	**1**	68	68.0
	2	27	27.0
	3	5	5.0
	Total	100	100.0

IAP Grading for PEM

1- Normal > 80%
2- Grade I 71 – 80%
3- Grade II 61 – 70%

68 children were – normal as per IAP Grading for PEM.

27 children fell under grade I PEM as per IAP Grading

5 children under Grade II PEM as per IAP Grading

WEIGHT AS PER IAP GRADING AND MEAN ZINC LEVEL

Report

ZINC

IAP	Mean	N	Std. Deviation
1	77.5103	68	21.7586
2	65.4222	27	28.8479
3	31.8000	5	20.8592
Total	71.9610	100	25.9002

1 – normal > 80%
2 – Grade 1 PEM
71 – 80%
3 – Grade II PEM
61 – 70%

P< 0.0001 (Sig)

Mean zinc level in children was in accordance with weight of the children corresponding to IAP classification. This association was statistically significant Pvale < 0.0001

Relationship of length / height with serum zinc level in accordance with Mc Laren classification.

	Frequency	Percent
1	87	87.0
2	13	13.0
Total	100	100.0

87 children were normal and 13 children were short statured

Grading as per Mclaren classification
1 – Normal - 93 – 105 %
2 – Short Stature 80 – 93 %

Report

LENGTH/ HEIGHT	Mean	N	Std. Deviation
1	74.9885	87	24.5225
2	51.7000	13	26.7090
Total	71.9610	100	25.9002

P < 0.002 (Sig)

P Value < 0.002

Mean zinc level in children was in accordance with their respective height/ Length which was statistically significant.

HEAD CIRCUMFERENCE IN RELATION TO SERUM ZINC LEVEL

	Frequency	Percent
1	25	25.0
N	75	75.0
Total	100	100.0

N - Normal head circumference
1 – HC – 1 Standard deviation below normal for the age

75 children were having normal head circumference and 25 were having head circumference one standard deviation below normal for the age

ZINC

HC CLASS	Mean	N	Std. Deviation
I	62.0920	25	25.8998
N	75.2507	75	25.2194
Total	71.9610	100	25.9002

P = 0.027 (sig)

Children with normal head circumference were having mean zinc level higher than children with head circumference one standard deviation below normal for the corresponding age.

This association was statistically significant with p value. 0.027.

SERUM ZINC LEVEL IN RELATION TO DEHYDRATION

		Frequency	Percent
Valid	1	56	56.0
	2	43	43.0
	3	1	1.0
	Total	100	100.0

1 – No dehydration
2 – Some dehydration
3 – Severe dehydration

56 children had no dehydration

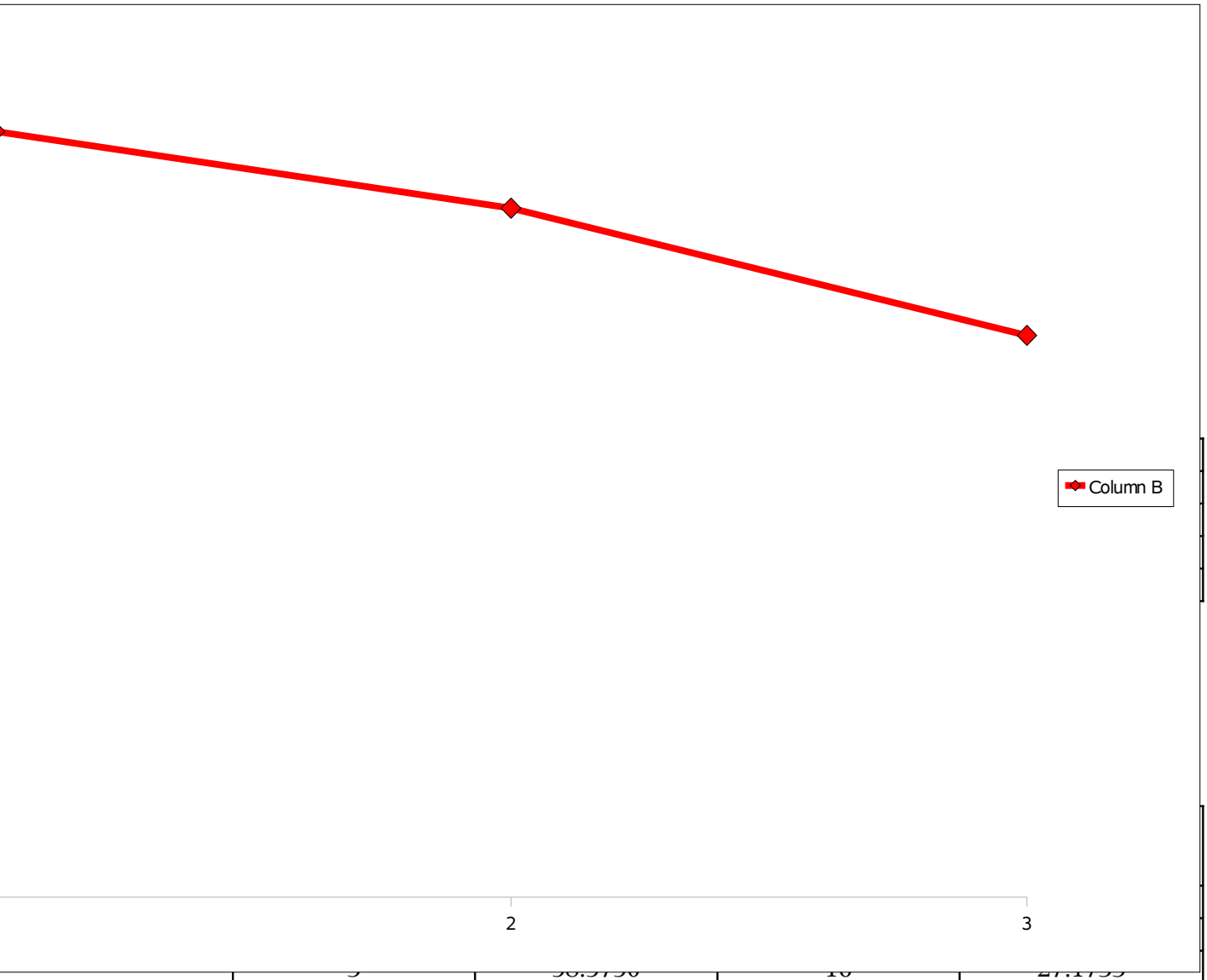
43 children had some dehydration

1 child had severe dehydration

ZINC

DEHYDRATION	Mean	N	Std. Deviation
1	71.2089	56	22.8325
2	71.8465	43	29.0703
3	119.0000	1	
Total	71.9610	100	25.9002

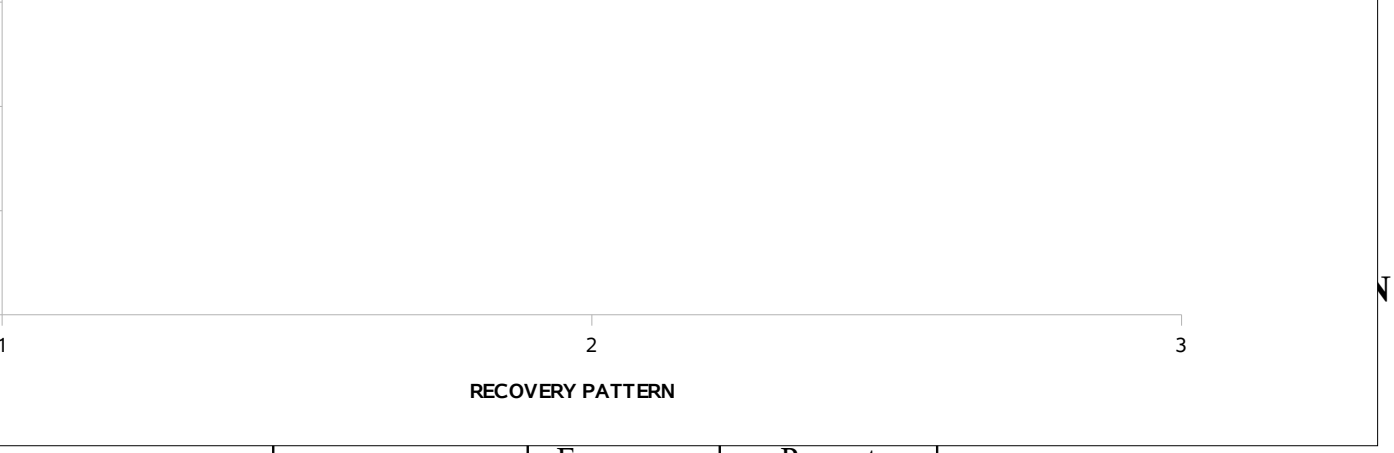
P = 0.1 Not significant



Total	**71.9610**	**100**	**25.9002**

P = 0.031 (Significant)

Low serum zinc level increases the frequency of loose stools as depicted by the P value being 0.031 and the association was statistically significant.



		Frequency	Percent
Valid	1	48	48.0
	2	43	43.0
	3	9	9.0
	Total	**100**	**100.0**

1 - Recovery ≤ 3 days

2 - Recovery 4 – 7 days

3 - Recovery > 7 days

48 children recovered from diarrhoea

43 children recovered from diarrhoea with in

9 children recovered from diarrhoea after 7 days

ZINC

RECOVERY PATTERN	Mean	N	Std Deviation
1	81.9292	48	22.7955
2	64.8512	43	23.9510
3	52.7667	9	30.8950
Total	**71.9610**	**100**	**25.9002**

P < 0.0005 – Significant

Children whose mean zinc level was higher had faster recovery pattern than children with low mean zinc level. Hence the association found out was statistically significant (P < 0.0005).

SERUM ZINC LEVEL IN RELATION WITH MODE OF FEEDING FOR THE FIRST 6 MONTHS :

	Frequency	Percent
1	12	12.0
2	82	82.0
3	6	6.0

1 - Exclusive breast feeding

2 - Breast feeding & cow's milk feeding

3 - Breast feeding + artificial

ZINC

FEEDING 1-6	Mean	N	Std Deviation
1	74.0500	12	26.0793
2	70.8829	82	25.9065
3	82.5167	6	27.3883
Total	**71.9610**	**100**	**25.9002**

P value 0.5

Through there was observed difference the P value was not statistically significant. Because the number of children among study groups were not evenly distributed.

SERUM ZINC LEVEL IN RELATION WITH COMPLEMENTARY FEEDS

Complementary Feeds

	Frequency	Percent
1	15	15.0
2	63	63.0
3	22	22.0
Total	**100**	**100.0**

1 - Started < 6 m

2 - Started between 6-7 months

3 - Started > 8 m

ZINC

Complementary Feeding	Mean	N	Std Deviation
1	78.4800	15	27.9079
2	74.8159	63	24.4900
3	59.3409	22	25.5112
Total	71.9610	100	25.9002

$P < 0.05$

The mean ZINC level was lower for those children for whom complementary feeding was started later. The association was statistically significant. (P Value < 0.05).

SOCIO ECONOMIC STATUS IN RELATION WITH SERUM ZINC LEVEL

	Frequency	Percent
1	6	6.0
2	86	86.0

Modified Kuppuswamy's Classification

1 - Lower Middle

2 - Upper lower

3 - Lower lower

3	8	8.0
Total	**100**	**100.0**

REPORT

ZINC

SE STATUS	Mean	N	Std Deviation
1	67.7333	6	19.0275
2	71.2977	86	26.5978
3	82.2625	8	22.2784
Total	**71.9610**	**100**	**25.9002**

P = 0.48 (NS)

P value 0.48

No Association could be made out between the serum Zinc level to socio economic status as the P value was 0.48; Hence the association was not statistically significant.

RESPIRATORY TRACT INFECTION IN RELATION WITH SERUM ZINC LEVEL

	Frequency	Percent
N	85	85.0
Y	15	15.0
Total	**100**	**100.0**

N – No
Y - Yes

ZINC

RTI	Mean	N	Std Deviation
N	73.8424	85	24.0122
Y	61.3000	15	33.7765
Total	**71.9610**	**100**	**25.9002**

P = 0.084 (Not significant)

From the analysis made, no relationship could be made out between the scrum Zinc level and respisatory tract infection since the P value is 0.084.

SERUM ZINC LEVEL IN RELATION WITH

LEVEL OF MOTHERS EDUCATION

	Frequency	Percent
1	8	8.0
2	45	45.0
3	36	36.0
4	9	9.0
5	2	2.0
Total	**100**	**100.0**

1 – illiterate
2 – upto calssV
3 – VI to X std
4 – XI to XII std
5 – degree holder

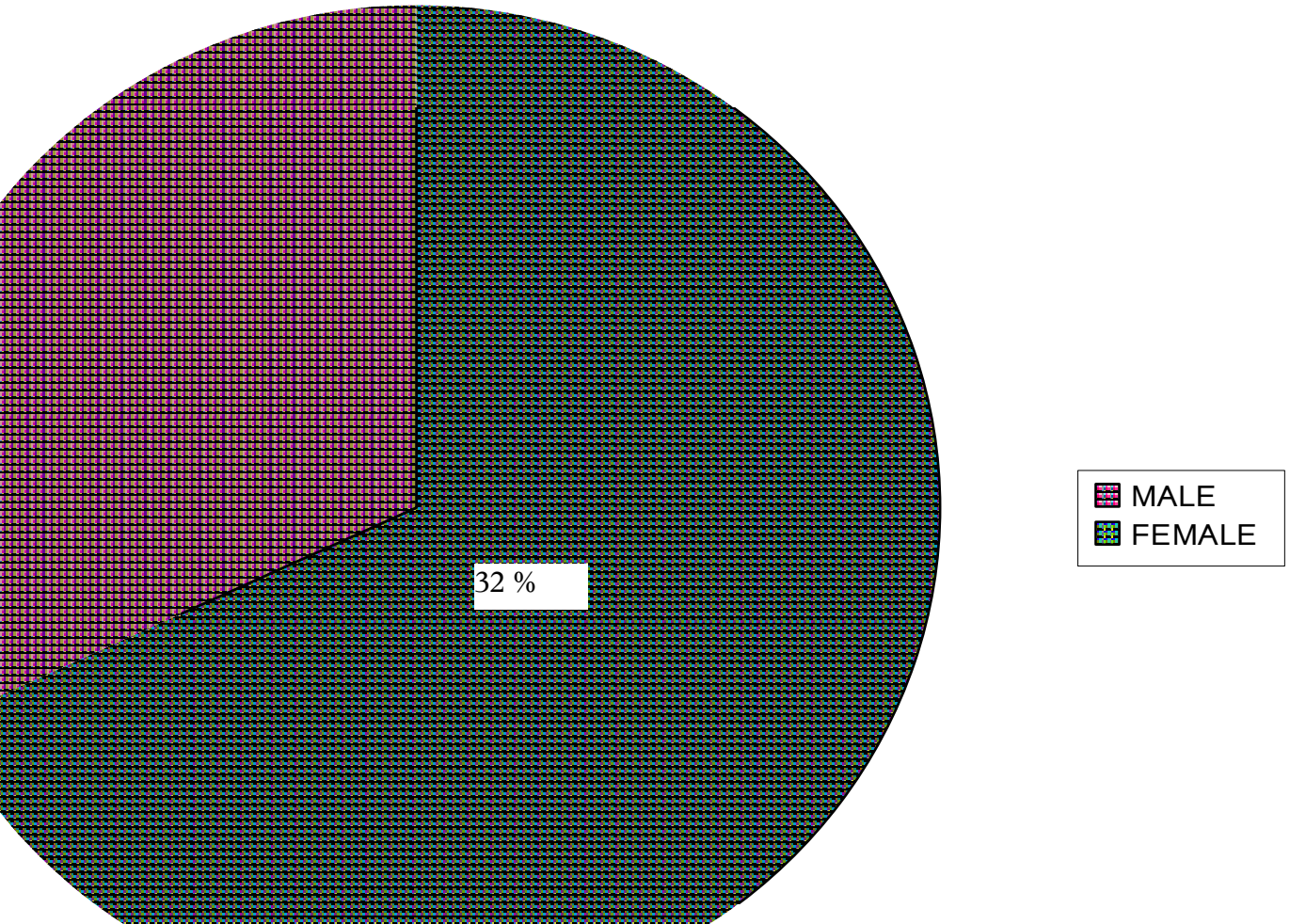
ZINC

MOTHER EDU	Mean	N	Std Deviation
1	83.8250	8	31.5769

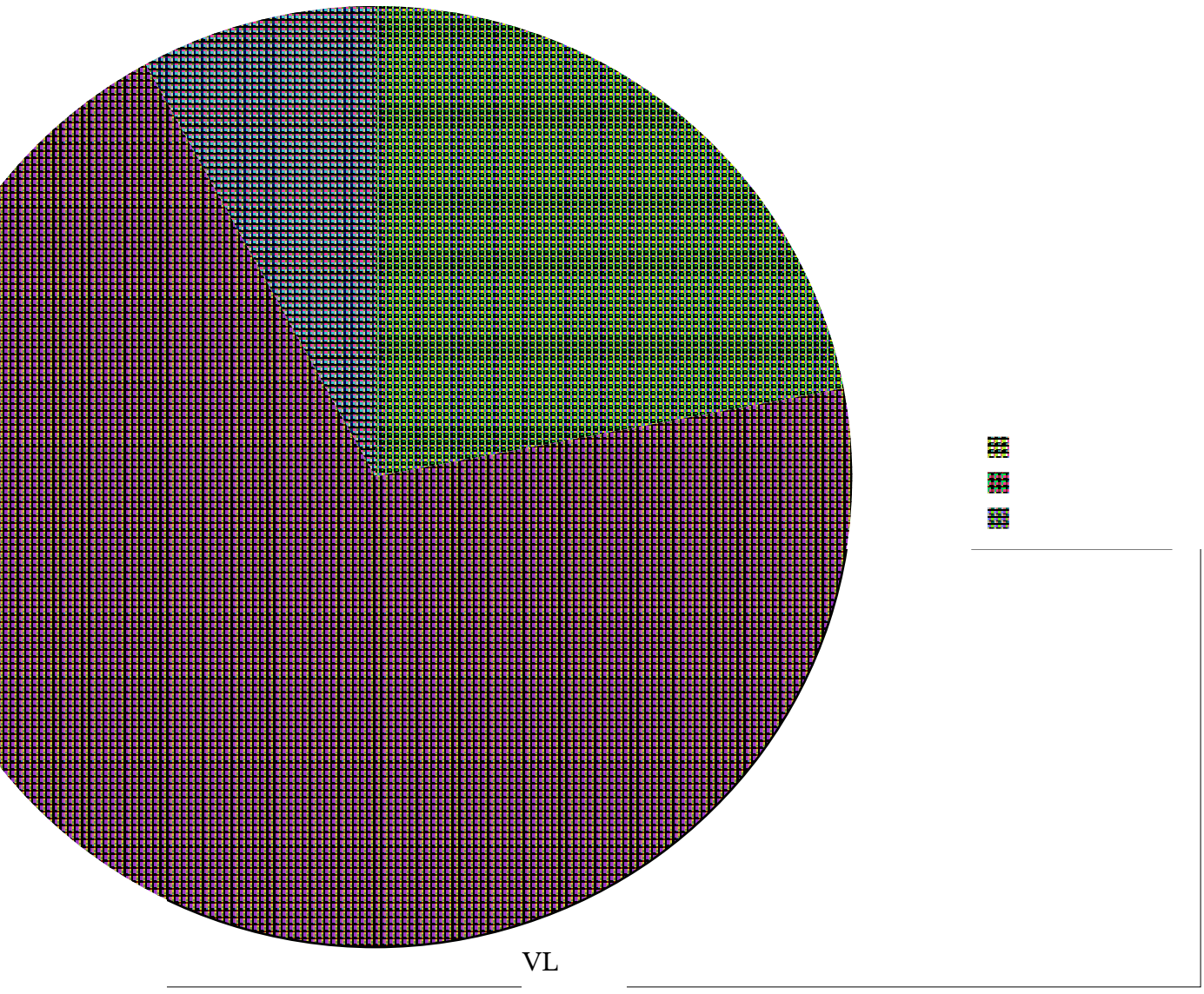
2	69.0267	45	22.4550
3	71.9000	36	28.9607
4	77.8333	9	26.1765
5	65.2000	2	22.2032
Total	**71.9610**	**100**	**25.9002**

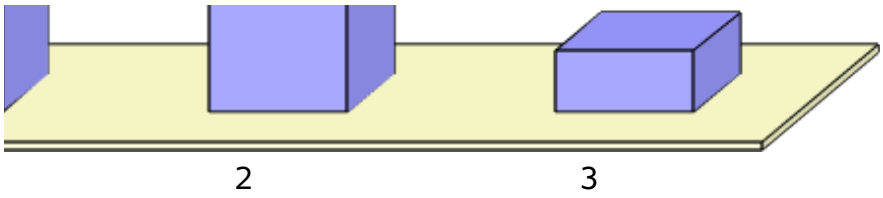
P = 0.59 (Not significant)

As the P value is 0.59, mother's education and the serum zinc level in the study group does not bear any statistical significance.



68 %





IAP CLASSIFICATION

OBSERVATION

The following observations were made from the detailed study which was conducted in RMH, Thanjavur.

Total number of study population was 100; among them 68 were males & 32 were females; No statistical significance was observed in their serum zinc levels.

The serum zinc level was low in 22% of the study subject and very low in 8% of them.

68 children were normally nourished; 27 fell under grade 1 PEM; 5 children under grade II PEM.

Mean zinc level was low of the value of 31.8 μg/dl of those with Grade II PEM and normal in those with normal nourishment.

Mean zinc level was low in children with short stature.

75 children of the observed population had normal head circumference and serum zinc level was low in those with head circumference one standard deviation below the normal value.

Observed difference in zinc level was found in those with normal hydration & those with severe dehydration.

Those with frequency of loose stools > 10 episodes / day had serum zinc level of 58.57 microgram /dl which falls under lower side of the normal.

serum zinc level reflected in the recovery pattern of diarrhoea and those with serum zinc level which is low of the value of 52.76 microgram / dl recovered very late, in our study 9 of them were observed to recover after 7 days.

22 children were started on complementary feeds after 8 months and they had mean serum zinc level of 59.34 µg/dl which was lower than normal.

Discussion :

Acute diarrhoeal diseases is one of the most common causes of under 5 yr mortality barring neonatal period; zinc deficiency is very common in developing countries and is more pronounced during an episode of diarrhea.

Normal serum zinc level is 60 -120 µg /dl (52, 53) and the p value of <0.05 was consided significant.

Serum zinc level was low in 30% of children in this study compared to 44% in the study conducted by department of international health Baltimore, maryland and international centre for diarrhoeal diseases and research Bangladesh. This was also studied by Ruz M, solomons NW & Hambidge K.M. (74.75).

Serum zinc level was low with mean zinc 49.89 µg in 22% of the study subject and was very low with mean zinc level of 24.5µg in 8% of children. The 'P' Value was <0.0005 and it was statistically significant.

No statistical association was made between serum zinc level and the sex of the children as the 'p' value was 0.065.

Zinc level and Anthropometry

Serum Zinc level was studied in relation to the standard anthropometric measurements. Weight was analysed in accordance with IAP grading for protein energy malnutrition. 68% were normal with

mean zinc level of 77.51 µg /dl; 27% fell under grade I PEM with` mean zinc level of 65.42% µg /dl and 5% fell under grade II PEM with mean zinc level of 31.80 µg /dl.

The association was statistically significant (P value < 0.0001)

This shows that serum zinc level was low in children with protein energy malnutrition. This is compatible with finding of Agget P.J. (1980) (76).

Mclaren's classification of length / height was taken as a criteria and the results studied in detail. 87 children were normal with mean zinc level of 74.98 µg /dl and 13 children were short statured and their mean zinc level was 51.7 µg / dl..

P value was <0.002; this was also statistically significant.

This observation was of the same findings of the study done in south India, Thiruvanandhapuram by Elizabeth, K.E.Sree Devi. The study showed that children mostly with PEM were in the post weaning period and most of the stunted children's zinc level were very low compared to others as also in study by Bhandari N, Bahl R, Taneja s.et al (77).

Head circumference was measured and its relationship to serum zinc was made out. HC was normal in 75% with mean zinc level of 75.25 µg /dl, 25% of children had their head circumference 1 standard deviation

below for age, and their mean zinc level was 62.92 µg /dl, and this association was also statistically significant with P value <0.027.

Serum zinc level and acute diarrhoea

Analysis was done to find out the association between the severity of dehydration and serum zinc level; no statistical association could be made out.

The relationship between serum zinc level and number of loose stools on the day of admission was also made out.

28 children had < 5 episodes and their mean Zinc level was 79.84 µg /dl; 56 children had 5 – 10 episodes with mean zinc level of 71.84 µg /dl, and 16 children had more than 10 episode of loose stools and their mean zinc level was 58.57 µg /dl.

This clearly depicts that low serum zinc level increases the frequency of loose stools and the association was statistically significant. (p value 0.031) This was also studied by Hetz and colleagues (78).

Serum zinc level and Recovery Pattern

48 of the study group fell under group I, who recovered from diarrhea in less than 3 days their mean zinc level was 81.92µg /dl.

43 children belonged to group II and recovered from diarrhea

within 4 – 7 days and their mean zinc level was 64.85 µg /dl. 9 children recovered after 7 days and their mean zinc level was 52.76 µg /dl.

This showed that those with lower zinc had prolonged recovery from diarrhea, compatible with findings of Tor Anne stand (79).

The effect of introduction of complementary feeds in relation to serum zinc level was studied.

Mean zinc level for whom complementary feeding started before 6 months was 98.78 µg /dl. And for those started between 6 – 7 months was 74.81 µg /dl. and for those after 8 months was 59.34 µg /dl.

This association was statistically significant with P value <0.05.

This shows that the time of introducing complementary feeds play a vital role in the level of zinc. This is compatible with study done by Dewey KG, Brown KH et al, (80).

The serum zinc level was analysed in relationship to the mode of feeding in the first 6 months only 12 children were exclusively breast fed and their mean zinc level was 74.05 µg /dl.

Though there was a difference among zinc level in each group, the association was statistically not significant.

The relationship between the socio economic status classified by modified kuppuswamy scale and the serum zinc level was made. 6 children fell under lower middle and 86 children were upper lower, 8 children under lower lower. The distribution of study groups were uneven and the association was not statistically significant.

Mother's education was the next parameter taken into account in studying the relationship between serum zinc level and acute diarrhea. No correlation could be made out and hence the association was not significant.

CONCLUSION :

Zinc deficiency is common in diarrhea. It's deficiency increases the no of loose stools & tends to prolong the recovery. To overcome this particular deficiency of trace element children should be exclusively breastfed, complementary feeds should be introduced at the appropriate time apart from other measures.

ORS has been used since time immemorial, even its use has not reduced the duration of stools and bulk of stools. To over come this obstacle and to overcome the deficiency of zinc in diarrhea, a more pragmatic and easy approach is oral supplementation of zinc as suggested by WHO and IAP which is as follows.

Zinc 20mg once daily should be given to all children with ADD of age >6 months for 14 days in addition to their standard treatment & zinc 10mg once daily should be given in children of 2-6 months of age for 14 days. It is of immense help by reducing the frequency of loose stools, improving the consistency & by hastening the recovery.

Making zinc easily accessible to the community and disseminating awareness among the health workers about the importance of zinc in the treatment of diarrhea will play a significant role in bringing down the mortality and morbidity in children.

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ANNEXURE – I

Consent

I _____ Parent/ Guardian of _____ have been told about the above study.

Being enrolled in this study will not change the original course of my child's treatment.

Being enrolled in the study will not harm my child's health, and it will not give me any financial advantage with regards to the treatment of my child. I am free to withdraw from the study if I choose to do so. Being aware of all of the above I give my consent for my child to be included in this study.

For any further details about the above, I may contact

Dr. X

Signature

Parent/ Guardian

Date :

ANNEXURE - II

PROFORMA

Name

Age in Months

Sex

Duration of Diarrhoea

Type of dehydration : No/ Some / Sereve

No of loose Stools / on the day of admission

Recovery pattern from diarrhea

Mode of feeding first 6 months

Complementary feeding
Started at what age

Mother's education

Socioeconomic status (as per modified kuppusamy's Scale)

Immunization

Anthropometry

Wt

Ht length

HC

Systemic Examination

CVS

RS

Abdomen

CNS